Original Article

MORPHOLOGICAL CHARACTERISTICS OF THE STOMACH MUCOSA IN CARNIVORES

Petko J. Zahariev,
Emil I. Sapundzhiev,
Despina V. Pupaki,
Pavel I. Rashev[1]

Dept. of Anatomy, histology and physiology,
University of Forestry,
Faculty of Veteriary Medicine,
Sofia
[1] Institute of Biology and Immunology of Reproduction,
Bulgarian Academy of Sciences

Corresponding Author:
Petko Zahariev
Dept. of Anatomy, histology and physiology,
University of Forestry,
Faculty of Veteriary Medicine,
10, Kl. Ohridski str.
Sofia, 1756
Bulgaria

Acknowledgements
The investigation was financially supported by the Grant 118/2008 of the University of Forestry

Summary
The stomach mucosa structure in animals belonging to Order Carnivora indicates some specific characteristics in comparison with the other mammals. Between the base of the mucosal glands and the lamina muscularis mucosae there is an additional plate which most of the morphologists have defined as lamina subglandularis. In currently used Nomina histologica this layer is indicated as stratum compactum in carnivorous stomach mucosa. The investigation aims were to study and compare canine and feline stomach tunica mucosa characteristics as well as to measure the thickness of stratum compactum and to specify some of the certain collagen types and fibronectin compounds. Conventional and differential histological and immunohistochemical methods for investigation of the canine and feline stomach slides were used. The specific organization of the stomach wall arrangement was established. In the structure of the canine stomach mucosa no evidence of stratum compactum was established. The presence of stratum compactum in feline stomach mucosa was ascertained and measured. In addition characterisation of the collagen types forming the mucosa of the investigated stomachs using specific antibodies was undertaken. The obtained results clarify the characteristics of the stomach mucosa morphology and could be used as a basis for distinguishing the stomach wall structure of the animal species belonging to Canidae and Felidae families although they are both carnivores.
Key words: stomach, mucosa, stratum compactum, canine, feline

Introduction
The carnivorous animals have a simple single-chambered stomach with a layered structure. In the specialized histological literature the presence of lamina subglandularis [1, 2, 3, 4] which is a specific peculiarity of the stomach mucous structure in carnivores is described. The information about its designation and compound shows a certain amount of discrepancies. Its presence and double-layered structure in the lamina propria mucosae underneath the fundus of the stomach glands has also been noted by Smollich A. [5], who however pointed it as stratum subglandulare. According to him, as well as others [3], the first layer which is situated closer to the bases of the glands is designated as stratum granulosum due to the fact that it is rich of connective tissue cells. The other layer which is positioned closer to the lamina muscularis mucosae is denser and is designated as stratum compactum. According to other authors [1, 2, 4] the lamina subglandularis is a single-layer dense fibrous acellular membrane which protects the stomach wall from

perforations. In the histological nomenclature Nomina histologica [5] it is noted that only one additional layer of the stomach mucosa in carnivores exists and it is named as stratum compactum. It is compound by collagen and is located between the bases of the mucous glands and the muscle lamina of the tunica mucosa. Frappier [7] also supports to this definition supplied by nomenclature as well as we maintain this opinion in our current studies [8].

The aims of the present study were to carry out a comparative morphological research of the stomach mucosa in carnivores from the Canidae and Felidae families, to measure the thickness of its stratum compactum and also to define the presence of certain collagen types and fibronectin in the structure.

Materials and Methods

Domestic bred dogs and cats unsuccessfully treated for various traumas or euthanized due to old age with the agreement of their owners were included in this study. A total of 12 dogs at the age between 3-14 years, 7 male and 5 female, as well as 8 cats at the age between 2 and 5 years old, 3 male and 5 female were studied. At the same time spontaneously the stomachs of 2 tigers a male and female, respectively from private circuses and 1 female fox shot after organized hunting, were used post mortem (Tab. 1). Biopsy was taken by partial gastrotomies from the stomach anatomic regions pars cardiaca, pars fundica and pars pylorica. The pets were under general narcosis and the ethical principles and legal requirements for the welfare of the animals were kept.

Table 1. Investigated carnivore animals.

Family	Species	Total (n)	Male (n)	Female (n)
Canidae	Dog *(Canis familiaris)*	12	7	5
	Fox *(Vulpes vulpe)s*	1	-	1
Felidae	Cat *(Felis silvestris domestica*	8	3	5
	Tiger *(Panthera tigris)*	2	1	1

The samples taken for histological investigation were fractionally fixed in 10% buffered formalin immediately after the gastrotomy. Histological slides for microscopic investigation were conventionally prepared. The tissue samples were embedded in paraffin wax and sections 7 µm thick were stained with hematoxylin and eosin. Differentially to distinguish the collagen in compound connective tissue the samples were proceeded through the dye-impregnation methods of Van Gieson and Heidenhain (modification method, such as Malory's).

Microscopic measurements were done with ocular-micrometer, calibrated on light microscope Olympus CX 21 and pictures and documentation were made on digital camera Olympus C5050 (Olympus Co Ltd., Tokyo, Japan).

For the immunohistochemical staining avidin-biotin-peroxidase complex method was used. The test sections were incubated with monospecific polyclonal antibodies against collagen types I, III, V and plasma fibronectin and monoclonal antibody against collagen type IV. Controls were incubated with PBS only. All steps were performed using Universal Elite ABC kit (Vectastain, Burlingame, CA 94010). The stepwise procedure is described elsewhere [8].

Results

The histological investigation of the stomach mucosa in carnivores belonging to the Canidae family has shown typical characteristics (Figure 1-1). In the course of the study upon the samples taken from the all anatomic areas of the canine stomachs there was surprisingly no evidence for the presence of stratum compactum in the tunica mucosa. Additional differential histological staining has also shown a lack of a compact collagen structure beneath the bases of the stomach glands. The morphological study of a fox stomach, the lack of the layer stratum compactum in its mucosa was once again registered.

During the investigation of the cat stomachs also from the registered anatomical areas the presence of the layer stratum compactum underneath the bases of the stomach glands was established as we had expected regardless of the different sex of the animals (Figure 1-2). The measured thickness of the stratum compactum in the domestic cat ranges between 32.5 and 37.5 µm. Its density and acellular structure is different from the other parts of the stomach mucosa. The conventional staining with hematoxylin and eosine revealed the eosionofilia of the collagen fibres. When differentiall stainig is applied according to the method of van Gieson the pink-red collagen fibres are densely situated just like a ribbon (Figure 1-3). When slides are stained according to the method of

Heidenhain, which is a modification of Malory's method, the collagen is stained in blue due to the used stain azan and the lamella-like structure of the layer is outlined (Figure 1-4).

After the pathoanatomical expertises of the two tigers (Panthera tigris) we determined the presence of the layer stratum compactum in the stomach mucosa with thickness ranging between 40 and 42.5 μm (Figure 1-5).

The immunohistochemical investigation of the collagen compound showed moderate expression of collagen type I in the stomach mucosa with dilution of the antibody 1:40 (Figure 1-6). Its distribution is even both in stratum compactum, as well as in the areas between the glands of lamina propria mucosae.

The staining for collagen type III in stratum compactum shows a stronger expression than the one of collagen type I as well as in areas of lamina propria mucosa underneath the bottoms of the stomach glands at the same dilution of the antibody 1:40 whereas in the subepithelial area of the mucosa the expression is weaker (Figure 1-7).

When tracing the expression of collagen type IV it was found that its display was most prominent in comparison to the other types of collagen. Even when the dilution of the antibody was 1:400 its display in stratum compactum still dominates in all anatomic areas (Figure 1-8). The expression of collagen type IV is also prominent in the subglandular part of the lamina propria mucosae and it becomes weaker in the areas between the bodies of the stomach glands and in the direction of the surface of the stomach mucosa.

The expression of collagen type V was positive with a moderate reaction similar to that of collagen type I also after using a dilution of the antibody 1:40 (Figure 1-9). The distribution of collagen type V in the mucosa is even and adequate to the

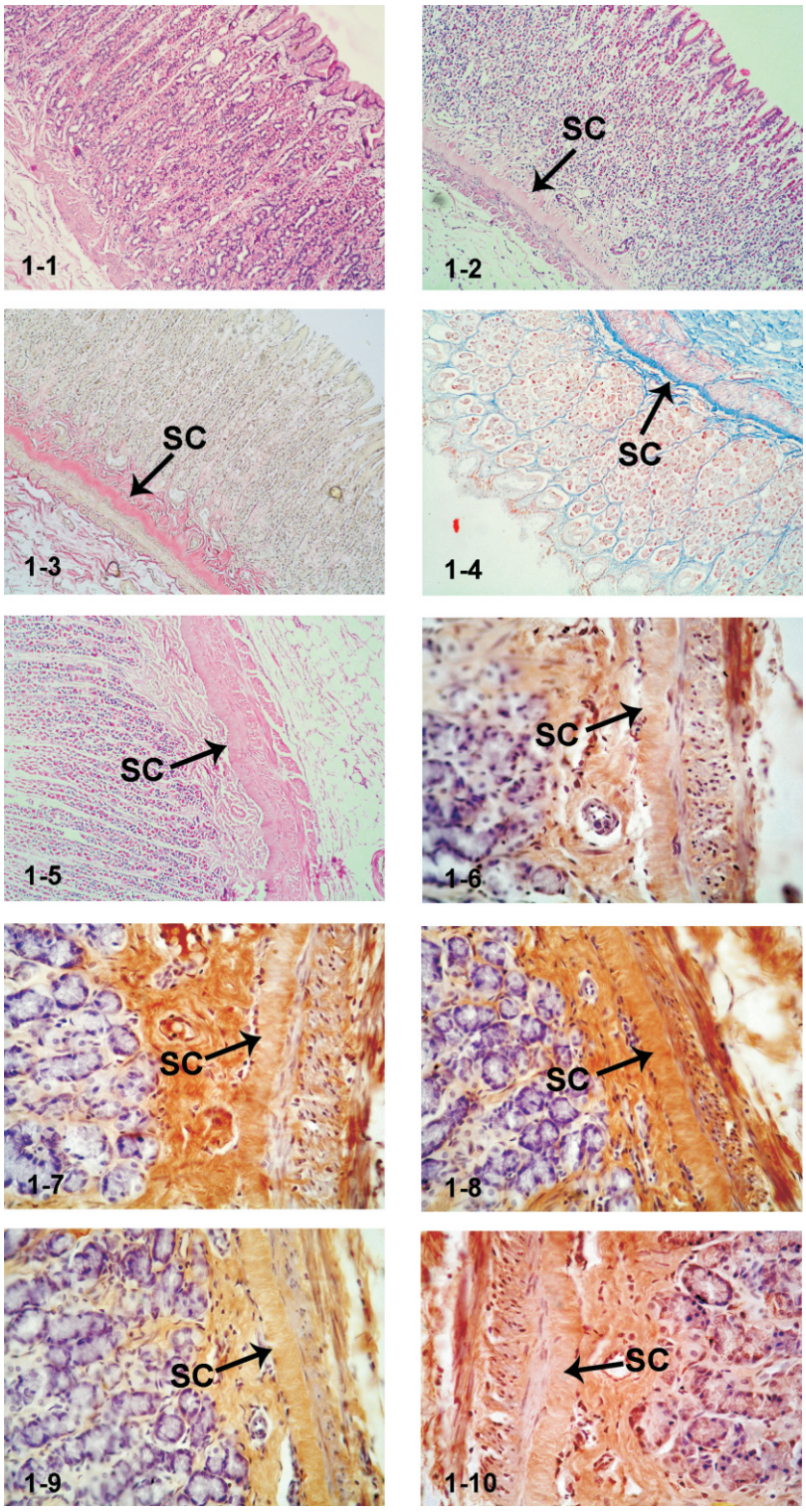

Figure 1. Morphological characteristics of carnivorous stomach mucosa. 1-1 mucous layer of dog stomach wall (H&E, 100X); 1-2 pars fundica of cat stomach wall (H&E, 100X); 1-3 pars fundica of cat stomach wall (Van Gieson, 100X); 1-4 pars fundica of cat stomach wall (Heidenhain, 100X); 1-5 tiger stomach wall (H&E, 100X); 1-6 collagen type I in cat stomach mucosa (400X); 1-7 collagen type III in cat stomach mucosa (400X); 1-8 collagen type IV in cat stomach mucosa (400X); 1-9 collagen type V in cat stomach mucosa (400X); 1-10 fibronectin in cat stomach mucosa (400X); SC stratum compactum.

localization of collagen type I.

The immunohistochemical staining for fibronectin with dilution of the antibody 1:400 has shown that the reaction is positive and well-expressed both in the layer stratum compactum as well as in the other mucous areas (Figure 1-10).

Discussion

The lack of stratum compactum in the structure of the canine stomachs mucosa, regardless of sex, age or breed, is a finding which is different from the other literature sources [2, 3, 4, 5, 6, 7]. The animals were over the age at which it is generally accepted that the organogenesis is completed and no further structural development would be expected. Only Bacha and Wood [1] note that dogs may be missing the lamina subglandularis which was the term previously used to designate the layer stratum compactum. Those authors do not specify any source of additional information and do not comment on the reasons and assumptions related to that fact. If the lack of stratum compactum in the mucosa of the canine stomach wall turns out to be an obligate characteristic this may become a distinguishing feature of this animal species when compared to other carnivores.

The information obtained is valuable in terms of clarifying the nomenclature characteristics of the canine stomach mucosa. In addition the question remains open since the lack of stratum compactum in the stomach mucosa is a characteristic feature for other species of the Canide family as well. In the case of a single finding in a shot fox belonging to this family the stomach wall was found to be missing a stratum compactum. In this connection the data from at least 6 separate animals should be processed in order for any statistical credibility to be stated. That is precisely why more detailed research of a bigger amount of animals from the Canidae family of a more diverse pattern regarding the breed and age is required.

The location and structure of stratum compactum corresponds to the information from the referred literature sources [5, 6, 7]. During our research no statistically significant difference in the thickness of the layer in cats of different age and sex was established. In the referred literature no information about the thickness of stratum compactum was shown which prevents us from making a comparative collation. The correlation dependency between the thickness of the layer and the size of the animal from the Felidae family is logical with confirming nature.

On the grounds of the stratum compactum presence only in cat and tiger stomach mucosa we decided to formulate the hypothesis that its presence is most probably the only characteristic of carnivores from the Felide family. A more extensive analysis of this data however requires future studies of more animal species from this family.

In the literature there is no information about the collagen types in the stomach mucosa of the cat as well as of other carnivores. Its main role in the architectonic of the intercellular matrix is defined by its importance as a fibrillar protein which is insoluble in water and the functional characteristics in the fibrous connective tissues [9]. So far over 25 types of collagen have been described among them 5 - 6 types have basic significance. It is known that collagen type I takes part in the formation of thick and strong bundles of fibres, which give the tissue its strength. That is why its moderate expression can be related to the postulate that the layer stratum compactum strenghtens the stomach wall.

The similar distribution of collagen types III and IV characterizes their functional necessity in the areas of the stomach mucosa, shown in this study. It is known that collagen III forms the reticular fibres, and its abundant expression can be important for the reticular distribution of the collagen.

Collagen type V is known to play a key role in the formation of the fibrillar collagen matrix and not to form any fibres. This is similar to the one of collagen type XI and is related to the fibrillogenesis of collagen and the formation of the collagen fibre core. On an electronic microscopic scale it has been determined that collagen type V takes part in the formation of basal membranes by situating itself in the lamella lamina densa [9]. This information gives us grounds to assume that stratum compactum has a structural organization similar to the structure of lamina basalis. Such an assumption needs to be substantiated by ultrastructural research in order to trace the location of collagen fibrils.

The intensive expression of fibronectin additionally characterizes the structure of stratum compactum. It is widely known that its globular molecule can be encountered in two forms- intracellular, as a metabolic product, and in the intercellular matrix, as an assistant for the adhesion of glycosaminoglycans and collagen fibres. A third function is recently being imparted to it, for which the most typical fact is the formation of plasmalemic epitopes necessary for the cellular recognition [10].

Conclusions

It has been specified that the structure of the stomach mucous membrane of animals from the Canidae family is different from the one of the Felidae family, even though they all belong to Carnivora order.

It has been determined that the dog and fox stomach mucosa does not include the layer stratum compactum which has been described in scientific literature.

The presence of the layer *stratum compactum* in the stomach mucosa of domestic and wild cats and tigers was confirmed as well as its thickness was measured by morphometry.

Using an immunohistochemical method it was shown very high expression of collagen type IV and fibronectin, moderate positive reaction of collagen type III, and a comparatively weakest expression of

collagen types I and V in the structure of *stratum compactum* from cat stomach mucosa.

References

1. Bacha WJr, Wood LM. Digestive system. In: Williams & Wilkins, editors. Color Atlas of Veterinary Histology, Media, PA, USA: Williams & Wilkins; 1990. p. 112-35.
2. Krystev H, Vitanov S. Simple stomach. In: G. Kondov, editor. Textbook of cytology and histology. Sofia: Zemizdat; 1993. p. 251-55. (in Bulgarian)
3. Stinson AW, Lois Calhoun M. Digestive system. In: Dellmann HD, editor. Textbook of Veterinary Histology (IV). Philadelphia: Lea & Febiger; 1993. p. 153-93.
4. Vitanov S, Dimitrov D, Bochukov A. Stomach. In: Popova M, editor. Manuel for practice of cytology and histology. Sofia: Zemizdat; 1995. p. 88-90. (in Bulgarian)
5. Smollich A. Magen. In: Sajonski H, Smollich A, editors. Mikroskopische Anatomie (in German). Leipzig: S. Hirzel Verlag; 1972. p. 166-88.
6. Nomina histologica. Histologia generalis. International Committee on Veterinary Histological Nomenclature; Gent: 1992. p. 15-37.
7. Frappier BL. Digestive system. In: Dellmann HD, Eurell J, editors. Textbook of Veterinary Histology (V). Baltomore, Maryland, USA: Williams & Wilkins; 1998. p. 164-80.
8. Sapundzhiev E, Zahariev P, Pupaki D. Morphologic investigation of the stomach mucosa characteristics in dogs and cats. Suppl. XVII Europ. Congress Vet. Anat., Budapest: 2008; (3) p. 32.
9. Hulmes DJ. Building collagen molecules, fibrils, and suprafibrillar structures. J. Struct. Biol. 2002;137(1-2):2-10.
10. Potts JR, Campbell ID. Structure and function of fibronectin modules. J. Matrix Bio. 1996;15:313-20.